CLAIMS

What we claim is:

1. A method of determining the quantity of a target RNA in a tissue sample, which comprises:

isolating the tissue sample from a host,

isolating total RNA from the tissue sample,

subjecting the isolated RNA to a reverse transcriptase reaction followed by a DNA polymerase amplification reaction using primers corresponding to transcribed sequences of the target RNA,

binding a labelled sequence corresponding to an internal transcribed sequence of the target RNA to amplified transcribed target RNA,

determining the amount of labelled sequence bound to amplified transcribed target RNA,

establishing an RNA standard of the number of copies of the target RNA, and

comparing the determined amount of bound labelled sequence in the sample to the RNA standard as a measure of the number of copies of target RNA in the tissue sample.

2. The method of claim 1 wherein said RNA standard is established by:

synthesizing an RNA molecule corresponding to the target RNA,

quantifying the synthetic RNA molecule,

effecting serial dilution of said synthetic RNA molecule to provide a plurality of samples of known starting copy number,

subjecting the synthetic RNA molecule in each sample to a reverse transcriptase reaction followed by a DNA polymerase amplification reaction using primers corresponding to transcribed sequences of the synthetic

RNA molecule and corresponding in sequence to those employed during DNA polymerase amplification of the transcribed sequence of the target RNA,

binding a labelled sequence corresponding to an internal transcribed sequence of the synthetic RNA molecule to amplified transcribed synthetic RNA molecule in each sample, said labelled internal sequence being the same as that uses to bind to amplified transcribed target RNA,

determining for each sample the quantity of labelled sequence bound to amplified transcribed target RNA, and

plotting the individual determination of the quantity of labelled sequence in each sample against the log of the known starting copy number for each of the samples to provide a plot.

3. The method of claim 2 wherein said comparison of the determined quantity of bound labelled sequence to the RNA standard is effected by regression analysis using said plot.

4. The method of claim 3 wherein said binding step is effected by hybridizing said labelled sequence to said amplified transcribed target RNA following said amplification step, and including the step of separating bound labelled sequence from unbound labelled sequence prior to said determination step.

5. The method of claim 4 wherein said label is radioactive.

6. The method of claim 3 wherein said binding step is effected by effecting said DNA polymerase amplification in the presence of said labelled sequence and said determination of the amount of bound labelled sequence

is effected by detecting the generation of a detectable label.

7. The method of claim 6 wherein said detectable label is fluorescence.

8. The method of claim 1 wherein said reverse transcriptase reaction and said DNA polymerase amplification reaction are both carried out using a single enzyme reaction.

9. The method of claim 8 wherein said single enzyme reaction is effected using *Thermus thermophilus* enzyme.

10. The method of claim 1 wherein said target RNA is a cytokine.

11. The method of claim 1 wherein said primers correspond to an internal transcribed sequence of the target RNA.

12. A method determining the quantity of a target RNA in a tissue sample, which comprises:

isolating the tissue sample from a host;

isolating total RNA from the tissue sample;

subjecting the isolated RNA to a reverse transcriptase reaction followed by a DNA polymerase amplification reaction (PCR) using primers corresponding to transcribed sequences of the target RNA;

binding a labelled sequence corresponding to an internal sequence complementary to one of the strands of the PCR product of the target RNA; and determining the amount of labelled sequence bound to amplified transcribed target RNA.

13. A method of quantifying more accurately a target RNA in a tissue sample, which comprises:

isolating the tissue sample from a host;

isolating total RNA from the tissue sample;

subjecting the total RNA to a reverse transcriptase reaction followed by a DNA polymerase amplification reaction using primers corresponding to transcribed sequences of the target RNA;

binding a labelled sequence corresponding to an internal sequence of the amplified product; and determining the amount of bound labelled sequence by the generation of a detectable label.

14. The method of claim 13 wherein the detectable label is a fluorescent tag.